DDW
MOLECULAR LABORATORIES

VALIDATION REPORT- USTAR NUCLEIC ACID POINT OF CARE EQUIPMENT UE4 DX SYSTEM AND THE EASY/NAT MALARIA TEST KIT

INTRODUCTION

In the face of emergence, re-emergence of infectious pathogens and challenges in the diagnosis of existing ones, there is increasing demands for development of accurate and rapid diagnostic tool for effective and timely detection of infectious agents. Nucleic acid-based technologies provide the needed approach and strategy in the understanding of pathogen-host relationship as well as pathogen identification.

Malaria infection is a parasitic infection mostly prevalent in sub-Sahara Africa. Four important species have been identified to plague humans namely: *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale* and *Plasmodium vivax. Plasmodium falciparum accounts* among the leading cause of death in pregnant women and the under-five.

The diagnosis of malaria in sun-Sahara Africa is faced with several challenges of which the availability of reliable and effective test kits and consumables, trained microscopists and sensitive testing equipment have been identified.

USTAR EASY/NAT DX SYSTEM AND THE EASY/NAT MALARIA TEST KIT.

USTAR EASY/NAT DX SYSTEM IS a point-of-care testing system based on three-stage magnetic conductivity extraction technology and patented Cross Priming Amplification Technology. The system is available in a 4, 8 or 16-module configuration. The EASY/NAT MALARIA TEST KIT has a malaria cartridge equipped with multiple hydrophobic separating layers to separate lysis buffer, washing buffer and reaction reagent.

VALIDATION REPORT

The validation of "Ustar Nucleic Acid Amplification and Detection Analyzer" using its associated Diagnostic kit for *Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae and Plasmodium vivax* DNA testing was done at the DDW laboratories.

End point Polymerase Chain Reaction was used to select 100 malaria positive and 100 negative samples as gold positive and negative standard respectively. The 23sRNA of the *Plasmodium* species were amplified by end point PCR using specific primers on and Eppendorf Mastercycler in a 30ul final volume. The PCRmix comprised of x1 master mix, 0.5ul of forward and reverse primers and 5ng of DNA extracted from the blood samples. The PCR conditions were as follows: initial denaturation 95°C, denaturation, 95 °C for 30s, annealing, 56 °C for 45s, extension: 68 °C for 1mn and final extension: 68 °C for 5min. The PCR products were resolved on a 1.5% agarose gel at 120V for 25min.

OLD POSITIVE STANDARD			
S/N	PCR	USTAR	PARASITE DENSITY
1	Positive	Positive	503/µL
2	Positive	Positive	2535/µL
3	Positive	Positive	1050/µL
4	Positive	Positive	975/µL
5	Positive	Positive	709/µL
6	Positive	Positive	5900/µL
7	Positive	Positive	3000/µL
8	Positive	Positive	2980/µL
9	Positive	Positive	500/µL
10	Positive	Positive	2010/µL
11	Positive	Positive	3780/µL
12	Positive	Negative	210/µL
13	Positive	Positive	7800/µL
14	Positive	Positive	6230/µL
15	Positive	Positive	790/µL
16	Positive	Positive	6750/µL
17	Positive	Positive	4030/µL
18	Positive	Positive	1237/µL
19	Positive	Positive	1640/µL
20	Positive	Positive	660/µL
21	Positive	Positive	760/µL
22	Positive	Positive	1005/µL
23	Positive	Positive	984/µL
24	Positive	Positive	745/µL
25	Positive	Positive	580/µL
26	Positive	Positive	610/µL
27	Positive	Positive	1063/µL
28	Positive	Positive	1020/µL
29	Positive	Positive	630/µL
30	Positive	Positive	587/µL
31	Positive	Positive	839/µL
32	Positive	Positive	765/µL
33	Positive	Positive	720/µL
34	Positive	Positive	830/µL
35	Positive	Positive	510/µL
36	Positive	Positive	935/µL
37	Positive	Positive	2540/µL
38	Positive	Positive	670/µL
39	Positive	Positive	540/µL
40	Positive	Positive	1010/µL
41	Positive	Positive	1735/µL
42	Positive	Positive	1635/µL
43	Positive	Positive	752/µL

44	Positive	Positive	617/µL
45	Positive	Positive	820/µL
46	Positive	Positive	4200/µL
47	Positive	Positive	3100/µL
48	Positive	Positive	5250/µL
49	Positive	Positive	432/µL
50	Positive	Positive	674/µL
51	Positive	Positive	736/µL
52	Positive	Positive	590/µL
53	Positive	Positive	619/µL
54	Positive	Positive	1005/µL
55	Positive	Positive	583/µL
56	Positive	Positive	672/µL
57	Positive	Positive	935/µL
58	Positive	Positive	567/µL
59	Positive	Positive	433/µL
60	Positive	Positive	480/µL
61	Positive	Positive	6867/µL
62	Positive	Positive	5080/µL
63	Positive	Positive	495/µL
64	Positive	Positive	675/µL
65	Positive	Positive	976/µL
66	Positive	Positive	768/µL
67	Positive	Positive	653/µL
68	Positive	Positive	825/µL
69	Positive	Positive	900/µL
70	Positive	Positive	1700/µL
71	Positive	Positive	403/µL
72	Positive	Positive	300/µL
73	Positive	Positive	6702/µL
74	Positive	Positive	555/µL
75	Positive	Positive	420/µL
76	Positive	Positive	300/µL
77	Positive	Positive	645/µL
78	Positive	Positive	455/µL
79	Positive	Positive	3200/µL
80	Positive	Positive	360/µL
81	Positive	Positive	775/µL
82	Positive	Positive	3006/µL
83	Positive	Positive	3600/µL
84	Positive	Positive	593/µL
85	Positive	Positive	745/µL
86	Positive	Positive	1110/µL
87	Positive	Positive	400/µL
88	Positive	Positive	643/µL
89	Positive	Positive	938/µL

90	Positive	Positive	343/μL
91	Positive	Positive	253/μL
92	Positive	Negative	304/μL
93	Positive	Positive	1350/μL
94	Positive	Positive	680/μL
95	Positive	Positive	753/μL
96	Positive	Positive	513/μL
97	Positive	Positive	303/μL
98	Positive	Positive	563/μL
99	Positive	Positive	470/μL
100	Positive	Positive	900/μL

GOLD NEGATIVE STANDARD		
S/N	PCR	USTAR
1	Negative	Negative
2	Negative	Negative
3	Negative	Negative
4	Negative	Negative
5	Negative	Negative
6	Negative	Negative
7	Negative	Negative
8	Negative	Negative
9	Negative	Negative
10	Negative	Negative
11	Negative	Negative
12	Negative	Negative
13	Negative	Negative
14	Negative	Negative
15	Negative	Negative
16	Negative	Negative
17	Negative	Negative
18	Negative	Negative
19	Negative	Negative
20	Negative	Negative
21	Negative	Negative
22	Negative	Negative
23	Negative	Negative
24	Negative	Negative
25	Negative	Negative
26	Negative	Negative
27	Negative	Negative
28	Negative	Negative
29	Negative	Negative
30	Negative	Negative
31	Negative	Negative
32	Negative	Negative
33	Negative	Negative
34	Negative	Negative
35	Negative	Negative
36	Negative	Negative
37	Negative	Negative
38	Negative	Negative
39	Negative	Negative
40	Negative	Negative
41	Negative	Negative
42	Negative	Negative
43	Negative	Negative
44	Negative	Negative

45	Negative	Negative
46	Negative	Negative
47	Negative	Negative
48	Negative	Negative
49	Negative	Negative
50	Negative	Negative
51	Negative	Negative
52	Negative	Negative
53	Negative	Negative
54	Negative	Negative
55	Negative	Negative
56	Negative	Negative
57	Negative	Negative
58	Negative	Negative
59	Negative	Negative
60	Negative	Negative
61	Negative	Negative
62	Negative	Negative
63	Negative	Negative
64	Negative	Negative
65	Negative	Negative
66	Negative	Negative
67	Negative	Negative
68	Negative	Negative
69	Negative	Negative
70	Negative	Negative
71	Negative	Negative
72	Negative	Negative
73	Negative	Negative
74	Negative	Negative
75	Negative	Negative
76	Negative	Negative
77	Negative	Negative
78	Negative	Negative
79	Negative	Negative
80	Negative	Negative
81	Negative	Negative
82	Negative	Negative
83	Negative	Negative
84	Negative	Negative
85	Negative	Negative
86	Negative	Negative
87	Negative	Negative
88	Negative	Negative
89	Negative	Negative
90	Negative	Negative

91	Negative	Negative
92	Negative	Negative
93	Negative	Negative
94	Negative	Negative
95	Negative	Negative
96	Negative	Negative
97	Negative	Negative
98	Negative	Negative
99	Negative	Negative
100	Negative	Negative

The sensitivity and specificity of the Ustar NAT and the PCR were computed using the formulae (Table 1) as prescribed by Baratloo *et al,* (2015).

Table1. Computation of sensitivity and specificity of test kits

	Gold Positive standard	Gold Negative Standard
Positive	True Positive**(A)**	False Positive**(B)**
Negative	False Negative**(C)**	True Negative**(D)**

Accuracy: A+D/(A+C)+(C+D)

Sensitivity: A/(A+C) × 100

Specificity: D/(D+B) × 100

Positive Predictive Value: A/(A+B) × 100

Negative Predictive Value: D/(D+C) × 100

Table2. Ustar performance on gold standard samples

	Gold Positive standard	Gold Negative Standard
Positive	98 (A)	0 (B)
Negative	2 (C)	100 (D)

Ustar Accuracy: A=98, D=100, C=2, B=0

98+100/(98+100)+(2+0)= 99%

Ustar sensitivity: A= 98, C=2

98/(98+2) x100= 98%

Ustar specificity: D =100, B=0

100/(100+0)x100=100%

Ustar Positive Predictive Value

98/(98+0)x100=100%

Ustar Negative Predictive Value

100/(100+2) x100=98%

Table3: Sensitivity, specificity, positive and negative predictive value of USTAR using end point PCR selected samples as gold positive and negative standards

	PCR vs USTAR
Accuracy	99%
Sensitivity	98%
Specificity	100%
Positive predictive value	100%
Negative predictive value	98%

The findings revealed that Ustar had an accuracy, sensitivity and specificity of 99%. 98% and 100% respectively.

It is worthy to note that the Gold Positive Standard selected by end point PCR that could not be detected by Ustar could be attributed the low blood sample volume (100ul) Ustar requires while 300ul of blood was used in end point PCR.

RECOMMENDATION

The performance evaluation of the Ustar Nucleic Acid Point of care equipment Ue4 Dx System and The Easy/Nat Malaria test Kit revealed that the system is highly sensitive and specific and have been found to perform satisfactory in clinical settings. Its introduction in line of strategies for the diagnosis of malaria will improve performance of the laboratory and reduce the numerous human errors and delays observed in malaria testing.

Alireza Baratloo, Mostafa Hosseini, Ahmed Negida , Gehad El Ashal. Simple Definition and Calculation of Accuracy, Sensitivity and Specificity. ***Emergency*** (2015); 3 (2): 48-49.

Dr. Sampson T. Parker
Director. DDW Molecular Laboratories

Prof. Tatfeng Mirabeau
Consultant Researcher for DDW